

2 copies of Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the developing Chicken Embryo-Report of the in-house investigations of Potassium Sorbate in the developing chicken embryo 2/3/75

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

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TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: February 3, 1975

THRU : Dr. Berbert Blumenthal, Acting Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D. *M. Jacqueline Verrett*
Reproductive Physiology Branch, HFF-157

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to
the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Potassium Sorbate
in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Potassium Sorbate

Protocol:

Potassium Sorbate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, ataxia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Potassium sorbate did not show any toxicity when administered via the air cell at 0 hours up to a level of 200 mg/kg, but air cell treatment at 96 hours was nearly 100% lethal at 100 mg/kg with a calculated LD₅₀ of 48.775 mg/kg (2.439 mg/egg). Yolk treatment at 0 hours was even more toxic with a calculated LD₅₀ of 0.8266 mg/kg (0.0413 mg/egg). Yolk treatment at 96 hours showed toxicity significantly above background between 50 and 100 mg/kg, but the slope of the regression line was not significantly different from zero ($p=0.05$).

There were a few scattered abnormal birds under all conditions of test but the abnormalities were not different from or higher in evidence than those of the solvent-treated or untreated controls. Potassium sorbate displayed no teratogenicity up to 200 mg/kg under the test conditions employed.

1. Potassium Sorbate, Lot #72683, Pfizer Co., New York, N.Y.
2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.
3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006.
4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

Potassium Sorbate

Air Cell @ 0 Hours

| Dose | | Number of Eggs | ** Percent Mortality | Percent Abnormal | |
|----------|---------|----------------------|-------------------------|---------------------|------------|
| mg/egg | mg/kg | | | Total | Structural |
| 10.00 | 200.000 | 110 | 33.63 | 6.36 | 1.81 |
| 5.00 | 100.000 | 109 | 34.86 | 2.75 | 1.83 |
| 2.50 | 50.000 | 110 | 25.45 | 1.81 | 0.00 |
| 1.25 | 25.000 | 110 | 33.63 | 6.36 | 0.90 |
| 0.50 | 10.000 | 110 | 18.18 | 2.72 | 0.00 |
| Water | | 130 | 33.07 | 1.53 | 0.76 |
| Controls | | 377 | 22.28 | 2.38 | 1.85 |

** Slope is negative

Table 2

Potassium Sorbate

Air Cell @ 96 Hours

| Dose | | Number of Eggs | ** Percent Mortality | Percent Abnormal | |
|----------|---------|----------------------|-------------------------|---------------------|------------|
| mg/egg | mg/kg | | | Total | Structural |
| 5.00 | 100.000 | 110 | 97.27* | 0.90 | 0.00 |
| 2.50 | 50.000 | 110 | 68.18* | 1.81 | 0.90 |
| 1.250 | 25.000 | 109 | 23.85 | 4.58 | 3.66 |
| 0.6250 | 12.500 | 110 | 27.27 | 0.90 | 0.90 |
| 0.250 | 5.000 | 109 | 25.68 | 5.50 | 1.83 |
| Water | | 138 | 33.33 | 2.89 | 0.00 |
| Controls | | 377 | 22.28 | 2.38 | 1.85 |

** LD₅₀ 48.7755 mg/kg (2.4387 mg/egg)

* Significantly different from solvent $p \leq 0.05$

Table 4

Potassium Sorbate

Yolk @ 96 Hours

| Dose | | Number of Eggs | ** Percent Mortality | Percent Abnormal | |
|----------|---------|----------------------|-------------------------|---------------------|------------|
| mg/egg | mg/kg | | | Total | Structural |
| 5.00 | 100.000 | 105 | 61.90* | 1.90 | 0.00 |
| 2.50 | 50.000 | 104 | 53.84* | 4.80 | 1.92 |
| 1.25 | 25.000 | 105 | 51.42 | 1.90 | 0.00 |
| 0.6250 | 12.500 | 105 | 66.66* | 3.80 | 1.90 |
| 0.250 | 5.000 | 105 | 48.57 | 3.80 | 1.90 |
| Water | | 114 | 38.59 | 1.75 | 0.87 |
| Controls | | 377 | 22.28 | 2.38 | 1.85 |

** Slope not significantly different from zero $p=0.05$

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Table 1

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| 5.00 | 100.000 | 109 | 34.86 | 2.75 | 1.83 |
| 2.50 | 50.000 | 110 | 25.45 | 1.81 | 0.00 |
| 1.25 | 25.000 | 110 | 33.63 | 6.36 | 0.90 |
| 0.50 | 10.000 | 110 | 18.18 | 2.72 | 0.00 |
| Water | | 130 | 33.07 | 1.53 | 0.76 |
| Controls | | 377 | 22.28 | 2.38 | 1.85 |

** Slope is negative

Table 2

Potassium Sorbate

Air Cell @ 96 Hours

| Dose | | Number of Eggs | ** Percent Mortality | Percent Abnormal | |
|----------|---------|----------------------|-------------------------|---------------------|------------|
| mg/egg | mg/kg | | | Total | Structural |
| 5.00 | 100.000 | 110 | 97.27* | 0.90 | 0.00 |
| 2.50 | 50.000 | 110 | 68.18* | 1.81 | 0.90 |
| 1.250 | 25.000 | 109 | 23.85 | 4.58 | 3.66 |
| 0.6250 | 12.500 | 110 | 27.27 | 0.90 | 0.90 |
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Potassium Sorbate

Yolk @ 0 Hours

| Dose | | Number of Eggs | ** Percent Mortality | Percent Abnormal | |
|----------|---------|----------------------|-------------------------|---------------------|------------|
| mg/egg | mg/kg | | | Total | Structural |
| 10.00 | 200.000 | 105 | 88.57* | 1.90 | 0.95 |
| 5.00 | 100.000 | 105 | 86.66* | 0.95 | 0.00 |
| 2.50 | 50.000 | 105 | 80.95* | 0.00 | 0.00 |
| 1.25 | 25.000 | 104 | 82.69* | 1.92 | 0.96 |
| 0.50 | 10.000 | 105 | 76.19* | 0.00 | 0.00 |
| Water | | 125 | 28.00 | 0.80 | 0.00 |
| Controls | | 377 | 22.28 | 2.38 | 1.85 |

** LD₅₀ 0.8266 mg/kg (0.04122 mg/egg)

* Significantly different from solvent $p \leq 0.05$

Table 4

Potassium Sorbate

Yolk @ 96 Hours

| Dose | | Number of Eggs | ** Percent Mortality | Percent Abnormal | |
|----------|---------|----------------------|-------------------------|---------------------|------------|
| mg/egg | mg/kg | | | Total | Structural |
| 5.00 | 100.000 | 105 | 61.90* | 1.90 | 0.00 |
| 2.50 | 50.000 | 104 | 53.84* | 4.80 | 1.92 |
| 1.25 | 25.000 | 105 | 51.42 | 1.90 | 0.00 |
| 0.6250 | 12.500 | 105 | 66.66* | 3.80 | 1.90 |
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